

PCT

DUPLICATE
WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau



(2A)

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : C12N 5/00	A1	(11) International Publication Number: WO 99/43785 (43) International Publication Date: 2 September 1999 (02.09.99)
---	-----------	---

(21) International Application Number: PCT/US99/04188 (22) International Filing Date: 26 February 1999 (26.02.99) (30) Priority Data: 60/076,273 27 February 1998 (27.02.98) US (71) Applicant: MEDICAL COLLEGE OF HAMPTON ROADS [US/US]; 601 Colley Avenue, Norfolk, VA 23507 (US). (72) Inventor: HODGEN, Gary, D.; 3844 Church Point Road, Virginia Beach, VA 23455 (US). (74) Agents: MEILMAN, Edward, A. et al.; Ostrolenk, Faber, Gerb & Soffen, LLP, 1180 Avenue of the Americas, New York, NY 10036 (US).	(81) Designated States: AU, CA, JP, NO, European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE). Published With international search report.
---	---

(54) Title: DERIVATION OF CELLS AND TISSUES FROM EMBRYONIC PRE-STEM CELLS FOR TRANSPLANTATION THERAPIES

(57) Abstract

A novel method of isolating and propagating a line of embryonic stem cells that originates from either morulae (pre-stem) or blastocyst (ICM stem cells) is disclosed for the purpose of transplanting cells, tissues or organs.

To: David Fay
Several individuals are
now trying to isolate ES cells.
from morulae or even earlier embryo
stages. Will you please
protect us?
[Signature]

**DERIVATION OF CELLS AND TISSUES FROM
EMBRYONIC PRE-STEM CELLS FOR TRANSPLANTATION THERAPIES**

Background of the Invention

5 The present invention relates to the derivation
of cells and tissues from embryonic pre-stem cells for
transplantation therapies.

Summary of the Invention

10 This invention relates to the use of dispersed
morula cells in preference to inner cell mass (ICM) from
blastocysts. The morula stage is the last pre-embryonic
stage without expression of any differentiation, making
these cells (pre-stem cells) all progenitors of embryonic
stem cells (ESCs) later present in blastocysts.
15 Conversely, the ICM from the blastocyst is already
differentiated from trophoblastic cells, which are by then
destined to become part of the placenta.

20 This invention also relates to the use of
chimeric introductions into pre-stem cell cultures and stem
cell propagations in culture. That is, "teacher-cells" or
spent media from them, that derived from other sources
(e.g. adults, cord blood, fetal tissues, etc.) will "teach"
undifferentiated pre-stem cells how to convert to our
sought-after therapeutic cell population both more rapidly
and more preferentially.

25 This invention also relates to the identification
and use of certain early markers of stem cell

- 3 -

"teacher cells" can be used to accelerate the propagation of the target stem cells.

5 In a preferred embodiment, the embryonic stem cells are cultured in a medium in the presence of at least one agent or cytokine in order to differentiate into target specific cells or tissues. Preferably, the agent or cytokine is selected from the group consisting of IL-1, TNF- α , IL-6, PTH, PDGF, PGE₂, cAMP, estrogens, anti-estrogens, progestins, anti-progestins, cortisol, GH, 10 androgens, I₃/T₃, VEGF and cyclosporin. Also preferably, the concentration of the agent or cytokine in culture medium is from about 1.0 pg/ml to about 10.0 ng/ml.

15 In another preferred embodiment, the target specific cells are selected from the group consisting of nerve cells, bone cells, immune cells, and pancreatic beta cells.

20 Techniques and parameters for the use of a broad spectrum of early stage metabolic markers are developed. Some such markers are, for example: Fe⁺⁺ sequestration, Hg accumulation, myeloid fibers, nerve growth factor, apoptotic factors, insulin synthesis, dopamine loading, hemoglobin loading, etc. Additionally, other early markers of embryonic stem cells can be identified.

25 Specific techniques are developed to demonstrate the foregoing. In one embodiment of the invention, embryonic stem (ES) cells are derived from either morula or blastocyst stage embryos by placing cells on fibroblast feeder layers. The colonies are evaluated for differentiation state using accepted markers. Further

- 5 -

from embryonic pre-stem cells for transplantation therapies. Specifically, growing-out of human blastocysts at a rate greater than 50% from the 2-cell stage of the pre-embryo should be achieved. Also, efficient harvesting of either morula stage pre-stem cells and/or stem cells isolated from the inner cell mass of blastocysts should be achieved. These embryonic pre-stem and stem cell populations should preferably remain viable in culture for more than one week.

10 Example 1

Clonal production of stem cells will be undertaken. These clones will respond to the ambient levels of glucose in their milieu, and in turn, insulin-dependent diabetes would be treated by transplanting these stem cell lines to served by a peripheral blood supply. the insulin secretory cells must accomplish renewal y propagation through mitogenic proliferation.

Example 2

Pluripotent stem cells will be isolated and directed to differentiate into hemopoietic destinies. Therefore, tissues derived from the blood cell group or beta cells of the immune response system will be replaced in deficient patients suffering from conditions such as HIV infection, post-chemotherapy, or irradiation depletion. Culture condition in vitro will direct the rate and degree of differentiation manifested by these pluripotent stem cells, such as the presence of "teacher cells" or certain additives to the media, e.g. cytokines.

Example 3

- 7 -

What is claimed is:

1. A method of isolating and propagating a line of embryonic stem cells that originates from either morulae (pre-stem) or blastocyst (ICM stem cells).

5 2. The method of claim 1, wherein the propagated line of embryonic stem cells are used for the purpose of transplanting cells, tissues or organs.

10 3. The method of claim 1, wherein at least one regulatory factor is used to propagate the line of embryonic stem cells.

 4. The method of claim 3, wherein the regulatory factor is derived from teacher cells or teacher cells' spent culture medium.

15 5. The method of claim 3, wherein the propagation is initiated by the formation of chimeric inner cell mass cells.

20 6. The method of claim 5, wherein the formation of chimeric inner cell mass cells comprises nuclear transplantation, mitochondrial substitution, or cytoplasmic depletion.

 7. The method of claim 1, wherein the embryonic stem cells are cultured in a medium in the presence of at least one agent or cytokine in order to differentiate into specific cells or tissues.

25 8. The method of claim 7, wherein the agent or cytokine is selected from the group consisting of IL-1,

- 9 -

15. The method of claim 1, wherein clonal properties of the propagated stem cells is achieved by adding at least one agent or cytokine to the culture medium to eliminate contaminating members of the stem cells that
5 did not properly differentiate, wherein the agent or cytokine is selected from the group consisting of IL-1, TNF- α , IL-6, PTH, PDGF, PGE₂, cAMP, estrogens, anti-estrogens, progestins, anti-progestins, cortisol, GH, androgens, I₃/T₃, VGEF and cyclosporin.

16. The method of claim 1, wherein the propagation of the line of embryonic stem cells is done *in vivo* by transplanting teacher cells into an area sufficiently close to the embryonic stem cells to allow for at least one regulatory factor made by the teacher cells to
contact the embryonic cells.

17. The method of claim 1, wherein the presence or absence of different concentrations of calcium is used to regulate the propagation of the line of embryonic stem cells.

18. The method of claim 1, wherein the propagated line of embryonic stem cells is grown in a three dimensional manner before being transplanted.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/04188

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	DINSMORE et al. Embryonic Stem Cells Differentiated In Vitro as a Novel Source of Cells for Transplantation. Cell Transplantation. 1996, Vol. 5, No. 2, pages 131-143, see entire document.	1-18
Y	SOTOMARU et al. A Comparative Investigation on the Potency of Cells from the Inner Cell Mass and Trophectoderm of Mouse Blastocysts to Produce Chimeras. Theriogenology. 1997, Vol. 48, pages 977-984, see entire document.	1-18
Y	UCHIDA et al. Effects of Feeder Cells and Growth Factors on the Proliferation of Mouse Primordial Germ Cells. Theriogenology. 1995, Vol. 44, pages 9-16, see entire document.	1-18